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Genetic mapping of powdery mildew resistance gene (*er*) in pea (*Pisum sativum* L.)

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Abstract

Genetic mapping of powdery mildew resistance gene (*er*) was carried out. Ten crosses were studied in the F_1 and F_2 to determine the map position of *Er* gene. The study confirmed linkage of *Er* with six other genes of linkage group 6, *viz.*, *Pl, Arg, Fl, Wlo, Na* and *ThiB*. The gene order revealed by this study is *Na–Wlo–Er–Arg–Fl–Pl–ThiB*.

Key words: *Pisum sativum*, powdery mildew resistance, linkage map

Introduction

Linkage maps of pea have been prepared based on morphological, physiological, pigmentation characters [1], isozyme markers [2], and a combination of morphological, isozyme and DNA markers [3, 4, 5]. Presently, pea is one of the most thoroughly studied crops among agricultural plants in terms of genetics and lags behind only maize. Besides being an object of wide scale genetic investigation, pea is an important grain legume grown worldwide for human consumption, animal feed, forage and green manure. The powdery mildew disease, caused by *Erysiphe pisi* can cause severe damage to pea, often acquiring epidemic proportions. A single recessive gene (*er*) governing resistance to powdery mildew was first reported by Harland [6], which was subsequently confirmed by several other researchers [7, 8, 9]. Conflicting reports on the chromosomal location of *Er* have appeared in the past, e.g. on chromosome 1 [10], 6 [5] and 7 [11]. However, two earlier reports were not found to be correct, and in the consensus linkage map of pea [5], *Er* was placed on chromosome 6. Though it was assigned to linkage group 6, linkage studies of *Er* were confined to a few loci, *viz.*, *Pl, P* and *Gty* of chromosome 6. Thus it is evident that the chromosomal location of this gene of immense commercial value has not been analyzed in sufficient detail. Therefore, with a view to have a more precise information on the chromosomal position of *Er*, mapping in relation to six morphological markers of chromosome 6, *viz.*, *Pl, Arg, Fl, Wlo, Na* and *ThiB* was taken up.

Materials and methods

Plant materials and crossing: Sixteen lines from the pea germplasm maintained at the Division of Genetics, IARI, New Delhi, were used to make ten crosses. The list of parents, their pedigree, and source of origin are listed in Table 1. The parental strains were crossed in the combinations: P 1746 × MD 1-24 (PC *er* 51), P 1744-1 × P 1760 (PC *er* 6), P 1743 × HFP 4 (PC 398), HFP 4 × P 1881 (PC 439), P 1744 × P1757 (PC 435), P 1442 × PG 3 (PC 400), P 1746-8-1 × Pusa 10 (PC 436), Pusa 10 × P 1760 (PC 441), P 1746-24-1 × P 1746-1-1 (PC 437), and P 1779-4 × P 1760 (PC15 J). It was assumed that reciprocal crosses would show no differences in the inheritance patterns as nuclear genes govern the traits under study.

Table 1. Origin of pea strains used in the investigation

Pea strain	Pedigree	Source
P 1746, P 1746-8-1, P 1746-24-1 and P 1746-1-1	Wt 11777	Poland
MD 1-24	MD 1-24	IARI, New Delhi
P 1744 and P 1744-1	Wt 10345	Poland
P 1760	L 179	I. C. Murfet, Tasmania, Australia
P 1743	Wt 10102	Poland
HFP 4	T 163 × EC 190196	HAU, Hisar
P 1881	SK 25	IARI, New Delhi
P 1757	NFB 754	John Innes Institute, UK
P 1442	IC 37255	Collection from Sikkim
PG 3	T 163 × Boneville	PAU, Ludhiana
Pusa 10	Early Superb × L 993	IARI, New Delhi
P 1779-4	F_4-716-3-2-10	IARI, New Delhi

Description of genetic markers: Black hilum is the phenotype of the gene *Pl*, Argenteum (*Arg*) is silvery hue on aerial parts, and *Fl* is air spotting on leaves. All the three phenotypes are conferred by dominant alleles. The recessive homozygotes of the gene *Wlo* cause waxlessness on upper surface of

leaves, *Na* results in extreme dwarfness, and *ThiB* is thiamine deficient.

Raising thiB and na mutants: Kumar and Sharma [12] isolated and characterized three thiamine deficient mutants, *viz.*, *ThiA*, *ThiB* and *ThiC*. Recessive homozygotes are called alboterminalis, as they die after a short period of growth and need exogenous thiamine feeding for survival. Thiamine hydrochloride 2 mg/ml is sprayed at regular intervals. The *nana* (*na*) mutant was discovered and mapped by Wellensiek [13]. He named the extra-dwarf mutant *nanus*, later it was changed to *nana* by Blixt [1]. The *nana* dwarf is a gibberrellic acid (GA) deficient mutant, and is maintained by exogenous application of 0.05 ppm GA.

Powdery mildew resistance screening: Since *Erysiphe pisi* is abundantly present in the fields, infection occurs from natural sources. The fungus is an airborne, obligate ecto-pathogen. The natural epidemic of the disease in the late sown pea crop at Delhi, coupled with artificial inoculation and infector rows facilitated error-free disease screening. To ensure infection, the F_2 materials were planted in December with two infector rows of the powdery mildew susceptible variety L 116 along the border. Spores from the diseased plants were also dusted on the F_2 plants. The infected surface of foliage (i.e. leaves and stipules) was totally covered with white powdery mass of fungus and the infection spreads to all aerial parts of the plant, including stem and pods of susceptible genotypes. Tissue beneath the infected areas in the susceptible plants turns brown, followed by the production of fruiting bodies called cleistothecia. In the resistant plants, infection was absent or localized to very small patches only on leaves and stipules, and never spreads to the stem, peduncle and pods. Observations on resistance/susceptibility were recorded in the field on individual F_2 plants. Due to complete dominance of susceptibility over resistance, the genotypes *ErEr* and *Erer* were indistinguishable.

Observations: As all the genes under study have visible effects on plant morphology, scoring was easy on the basis of the character in the parents and segregants.

Statistical analysis: The χ^2 test [14, 15] was used for detection of linkage between genes. Once linkage is established, its intensity was estimated as recombination frequency (p) by the Product Ratio method and the Kosambi's mapping function [16] was applied to derive distances in cM from the estimated values of 'p'. The data on F_2 segregation of DD : Dr : rD : rr type were pooled.

Results and discussion

There was no plant absolutely free from infection. Even the resistant genotypes received mild infection. In the resistant plants, however, the sparse fungal growth was restricted to foliage without browning of the tissue affected. In contrast, all aerial parts including stem and pods of the susceptible plants were completely covered with fungus and the tissue beneath turned black. The reaction of parents to powdery mildew was known even before the present investigation was undertaken. All F_1 hybrids were susceptible to powdery mildew, which confirms dominance of susceptibility over resistance. Likewise, dominance of black hilum (*Pl*), silvery hue (*Arg*), and air spotting (*Fl*) and recessive nature of waxlessness (*wlo*), extreme dwarfism (*na*), and thiamine deficiency (*thiB*) was confirmed by observations in the F_1 generation.

All the traits under study had monogenic inheritance (Table 2) with a good fit to 3:1 ratio. The analysis of 3135 F_2 plants in 10 crosses confirmed monogenic inheritance of powdery mildew resistance [9]. Being discrete characters with monogenic inheritance, the phenotype of individual plants was observed without ambiguity.

Table 2. F_2 segregation on genes of chromosome 6

Gene	Cross(es)	No. of F_2 plants with phenotype			χ^2 (3:1)	Probability (P)
		DD/Dr	rr	Total		
Er	Pooled over 10 crosses	2356	779	3135	0.3	0.90
Pl	Pooled over PC er 51, PC er 6, PC 435, PC 437, PC 400, PC 441 and PC 436	1589	543	2132	0.0004	>0.9
Arg	Pooled over PC er 51 and PC 437	256	95	351	0.006	>0.9
Fl	PC 398	307	105	412	0.0003	>0.9
Wlo	Pooled over PC er 6, PC 435, PC 436 and PC 15 J	667	231	898	0.0009	>0.9
Na	Pooled over PC er 6, PC 441 and PC 15 J	474	157	631	0.00009	>0.9
ThiB	PC 439	283	93	376	0.0001	>0.9

Note: F_2 segregation designated by dominant (D) and recessive (r) phenotype of the gene.

The joint segregation analysis for gene pairs is presented in Table 3. The linkage χ^2_L was significant for all the gene pairs studied except for *Pl–wlo* and *Pl–na*. Pairwise analysis to determine map distance was also done in the pooled data in coupling and repulsion phases for the crosses. The linkage intensity varied from 23 to 38.5% for the gene pair *Er–Pl* in coupling phase (three crosses) and in repulsion phase (four crosses) (Table 3). The pooled analysis yielded the map distance of 39.6 cM in coupling phase and

Table 3. Joint F_2 segregation of genes *Er, Pl, Arg, Fl, Wlo, Na* and *ThiB*

Gene Pair	Cross No.	Phase	No. of F_2 plants with phenotype					Joint χ^2_L	Recombination frequency (%)	S.E (%)	Kosambi's map units (cM)
			Total	DD	Dr	rD	rr				
Er – Pl	PC er 51	Coupling	223	137	33	28	25	15.9	32.5	2.7	38.7
Er – Pl	PC 435	Coupling	219	127	36	34	22	10.4	38.5	2.9	50.9
Er – Pl	PC 437	Coupling	128	83	15	11	19	26.8	23.0	2.9	24.8
Er – Pl	**Pooled**	**Coupling**	**570**	**347**	**84**	**73**	**66**	**43.0**	**33.0**	1.7	**39.6**
Er – Pl	PC 441	Repulsion	253	132	56	61	4	16.9	25.5	3.9	28.1
Er – Pl	PC er 6	Repulsion	163	89	31	38	5	3.6	36.5	4.5	46.4
Er – Pl	PC 400	Repulsion	845	439	198	180	28	25.8	35.2	2.0	43.7
Er – Pl	PC 436	Repulsion	301	162	64	68	7	11.9	32.0	3.4	37.8
Er – Pl	**Pooled**	**Repulsion**	**1562**	**822**	**349**	**347**	**44**	**53.8**	**35.5**	1.5	**44.3**
Er – Arg	PC er 51	Repulsion	223	112	58	49	4	11.7	25.5	4.1	28.1
Er –Arg	PC 437	Coupling	128	85	13	10	20	33.3	20.0	2.7	21.0
Er – Fl	PC 398	Repulsion	412	216	94	91	11	15.5	32.5	2.9	38.7
Er – Wlo	PC er 6	Repulsion	163	76	44	42	1	20.4	15.2	5.1	15.6
Er – Wlo	PC 435	Repulsion	219	113	50	55	1	18.9	15.2	4.4	15.6
Er – Wlo	PC 15 J	Repulsion	215	108	55	51	1	20.8	13.5	4.5	13.8
Er – Wlo	**Pooled**	**Repulsion**	**597**	**297**	**149**	**148**	**3**	**59.8**	**14.0**	2.7	**14.3**
Er – Wlo	PC 436	Coupling	301	198	28	24	51	92.6	19.0	1.7	19.9
Er – Na	PC er 6	Coupling	163	104	16	19	24	15.5	24.5	2.7	26.8
Er – Na	PC 441	Coupling	253	158	30	35	30	23.8	30.5	2.4	35.3
Er – Na	PC 15 J	Coupling	215	139	24	19	33	48.7	22.0	2.2	23.6
Er – Na	**Pooled**	**Coupling**	**631**	**401**	**70**	**73**	**87**	**100.4**	**26.0**	1.4	**28.7**
Er – Thi B	PC 439	Repulsion	376	201	80	82	13	8.3	37.0	2.7	47.5
Pl – Arg	PC er 51	Repulsion	223	104	61	57	1	32.9	12.0	4.4	12.6
Pl – Arg	PC 437	Coupling	128	91	3	4	30	100.3	5.5	1.4	5.7
Wlo – Na	PC er 6	Repulsion	163	79	39	44	1	17.7	14.5	5.1	14.9
Wlo – Na	PC 15 J	Repulsion	215	108	53	53	1	20.9	13.5	4.5	13.8
Wlo – Na	**Pooled**	**Repulsion**	**378**	**187**	**92**	**97**	**2**	**38.5**	**14.0**	4.5	**14.3**
Pl – Wlo	PC er 6	Coupling	163	94	33	24	12	0.65 (ns)			
Pl – Wlo	PC 435	Repulsion	219	127	40	47	4	0.85 (ns)			
Pl – Wlo	PC 436	Repulsion	301	184	53	52	12	0.19 (ns)			
Pl – Na	PC er 6	Repulsion	163	95	32	28	8	0.11 (ns)			
Pl – Na	PC 441	Coupling	253	147	46	46	14	0.005 (ns)			

Note: F_2 segregation designated by dominant (D) and recessive (r) phenotype of the first and second trait in each gene pair; ns–non significant χ^2_L value.

44.3 cM in repulsion phase. The results of similar exercise for other gene pairs are also presented in Table 3.

The estimates of linkage for a given gene pair vary in different crosses, which is a consequence of chromosomal rearrangements [17, 18]. The differences in linkage intensities can also be due to the phase in which the alleles exist in the parents involved in different crosses.

The cross PC *er* 51 segregated for three loci, *Er, Pl* and *Arg.* The estimates of genetic distances obtained are *Er–Arg* 28.1 cM, *Er–Pl* 38.7 cM, and *Arg–Pl* 12.6 cM. Thus, the order of genes is *Er–Arg–Pl.* The value obtained directly between *Er* and *Pl* (38.7 cM) is very close to the sum of the other two intervals, i.e., *Er–Arg* and *Arg–Pl.* The arrangement of genes obtained from different crosses is presented in Fig 1. The order of genes derived by putting all the segments together is *Na–Wlo–Er–Arg–Fl–Pl–ThiB*. The present study convincingly maps the *Er* gene on chromosome 6 in relation to six known morphological markers of this chromosome. Earlier, *Er* was mapped only in combination with three markers of chromosome 6, i.e., *Pl, P* and *Gty* [5, 8, 19]. The map positions of *Fl* and *ThiB* are shown based on the earlier reports of their linkage with the *Pl* gene [5, 12] while the present estimates of their distances are from *Er.* The gene order generally matches with the consensus map [5]. The only exceptions are the positions of *Wlo* and *Fl.* In our study, *Wlo* mapped between *Na* and *Er,* while in the consensus map *Na* is between *Wlo* and *Er.* Such situations can be explained by assuming that the parent strains used in different studies could differ in

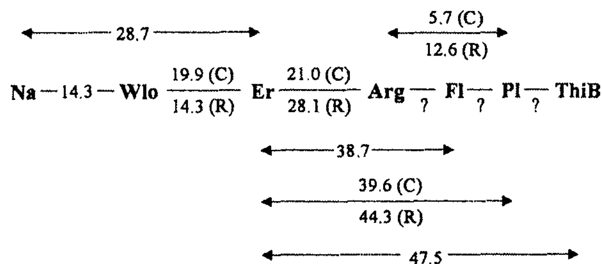

Fig 1. Gene map of chromosome 6 of pea. Genetic distance in CM. C—coupling phase, R—repulsion phase

gene arrangements due to chromosomal rearrangements in their evolution [18]. This could also be the cause of highly variable recombination values that are pooled to derive a single value for the consensus map. Similarly, the position of *Fl* (*Arg–Fl–Pl*) revealed in our study does not match the consensus map (*Arg–Pl–Fl*). Joint segregation of *Fl* was studied only with respect to *Er* and not with any other marker of chromosome 6. The gene order *Arg–Fl–Pl* is proposed because *Er–Fl* distance 38.7 cM is less than the *Er–Pl* value 39.6 cM in coupling phase and 44.3 cm in repulsion phase. It must be, however, noticed that the map values for the *Er–Fl* and *Er–Pl* distances are high and their segregation in the F_2 almost reaches the level of independent assortment. Under such situation minor discrepancies in gene order based on phenotypic segregation cannot be ruled out. A more detailed mapping in the *Er–Fl–Pl* region will confirm the position of *Fl*.

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